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BASIC FUCHSIN AS A STAIN FOR OSMIUM-FIXED, EPON-EMBEDDED TISSUE

As an aid for orientation, 1 μ sections taken adjacent to the ultrathin sections used in electron microscopy can be stained and examined by light microscopy as morphological references. Houck and Dempsey (1954), Jennings *et al.* (1959), and Runge *et al.* (1958) have developed staining techniques for osmium-fixed, methacrylate-embedded tissue which facilitate the examination of such reference sections. Toluidine blue has been used by Trump *et al.* (1961) to stain 1 μ sections of osmium-fixed, epoxy resin-embedded tissue, but it fades in less than a week and is difficult to use.

Procedure. The kidney of a rat was fixed by perfusion with Dalton's chrome-osmic fixative. Small samples of the fixed tissue were then routinely embedded in epoxy resin and sectioned. Sections 0.08 μ thick were stained with uranyl acetate and examined under an electron microscope with a 1 mil aperture. Adjacent sections 1.0 μ thick were placed on a drop of 10% acetone on a glass slide, dried on a hot plate and stained as follows:

1. Soak in distilled water for 5 min.
2. Stain in 1% basic fuchsin in 50% acetone for 7 min.
3. Wash twice in distilled water, 5 min each.
4. Dehydrate in graded ethanol to absolute and clear in xylene; 0.5–1 min total elapsed time.
5. Mount in a synthetic resin (Permount was used).

The intensity of the stain decreases during dehydration (step 4), therefore treatment time in the series of ethanols may be varied to give the desired staining.

A portion of a 1 μ section stained with basic fuchsin and photographed by light at a magnification of 2,200 is shown in figure 1. Figure 2 is an electron micrograph of a 0.08 μ section taken adjacent to that of figure 1. In figure 1 mitochondria, nuclei, and basement membrane are stained, but epoxy resin present in the tubule lumen is unstained. The tissue detail corresponds closely in the two figures. This use of basic fuchsin represents a simple, fast and reliable method for light microscopy of osmium-fixed, epoxy resin-embedded tissue.—JERRY WINKELSTEIN[1] and MAX G. MENEFEE, *Department of Anatomy*,

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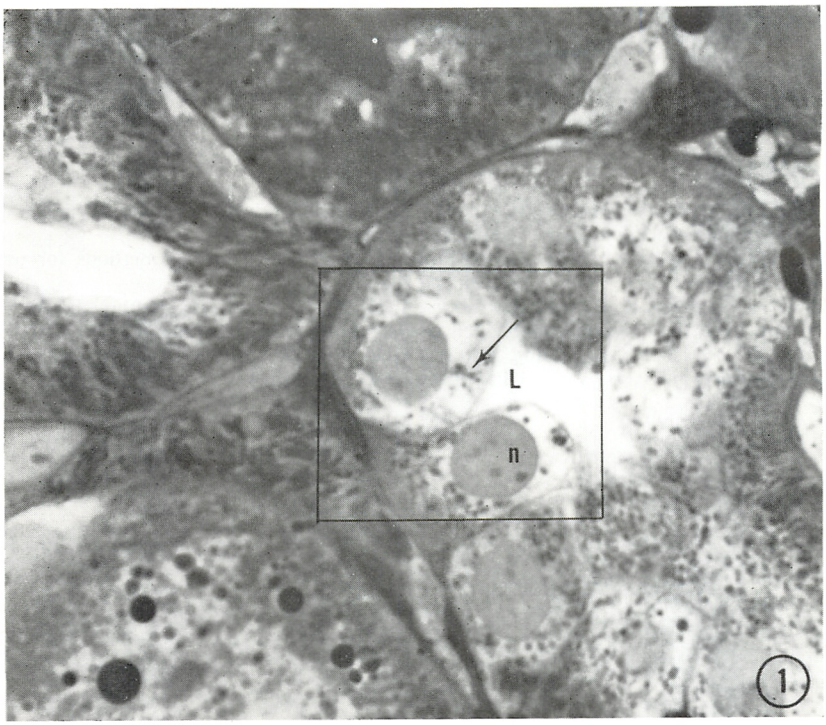

FIG. 1. Light micrograph of a 1 μ basic fuchsin-stained section of rat kidney fixed with Dalton's fixative and embedded in epoxy resin. Note staining of mitochondria (arrow), nucleus (n) and lack of stain in tubule lumen (L). $\times$ 2,200.

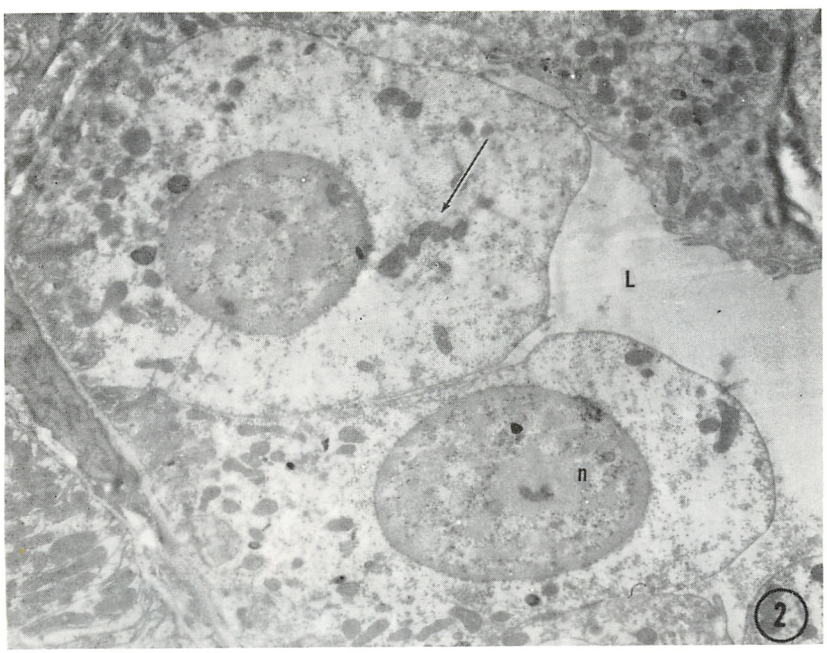

FIG. 2. Electron micrograph of an 0.08 μ section adjacent to Fig. 1 including the area within the rectangle. The structures labelled correspond to those labelled in Fig. 1. $\times$ 6,600.

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